Cyclic Mechanical Strain Guides Capillary-Like Morphology *in Vitro*

D. Koennig[1-4], C.J. Wilson[1-2], G. Kasper[1,3], S. Werner[1], K. Lehmann[1,3], G.N. Duda[1-4]

1. Julius Wolff Institut, Charité - Universitätsmedizin, Berlin
2. Center for Musculoskeletal Surgery, Charité – Universitätsmedizin, Berlin
3. Berlin-Brandenburg Center for Regenerative Therapies, Berlin
4. Berlin-Brandenburg School for Regenerative Therapies, Berlin

INTRODUCTION

Stretching of tissues stimulate angiogenesis [1,2] but increased motion at the site of a bone fracture hinders revascularisation [3,4].

Hypothesis: Cyclic tension applied during endothelial network assembly results in an increased formation of vascular structures, up to a threshold defined by mechanical disruption of cell–cell or cell–matrix adhesion (cellular adhesion strength).

MATERIALS AND METHODS

Assay:

- Angiogenesis assay (n = 3) based on fibroblast/HUVEC co-culture devised by Bishop et al. [5].
- Triplicate samples in fibronectin-coated BioFlex plates (FlexCell International) with cyclic equi-biaxial strain at 1Hz, 6 hours/day, via the FlexerCell FX-4000T system (FlexCell) (fig.1), for 7 days.

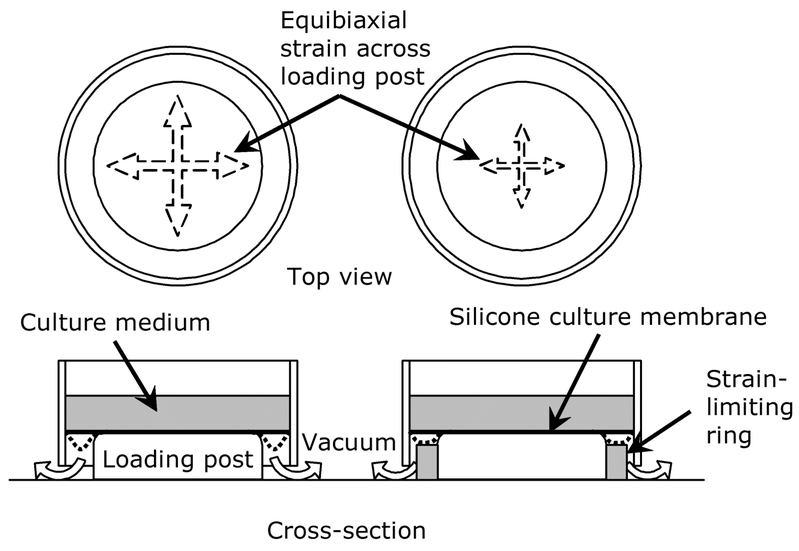

Figure 1: Schematic of modified FlexerCell well configuration.

- Loading stations modified with a series of limiting rings [6], allowing simultaneous application of multiple strain magnitudes.
- Resulting mean strains across the controlled central region of each well, at the maximum (for 1Hz) set-point of 12%: 1.0%, 4.4%, 7.4%, 10.1%, 13.2% (+one well blocked as static control).

Labeling, analysis:

- Mouse anti-human PECAM-1 (clone 9G11), detected with HRP-conjugated goat anti-mouse IgG(H+L), stained using a DAB kit.
- Microscopic images thresholded, skeletonised and analysed using a custom-written macro for ImageJ.

Output:

- Length of each endothelial network and individual segment and number of junctions (after post-processing data to remove branches and objects shorter than one cell length).

Statistics:

- Student's *t*-test for paired samples for each parameter; ANOVA to test variation in network length with strain magnitude.

RESULTS

Simultaneous co-cultures

Endothelial network formation in co-culture under cyclic strain

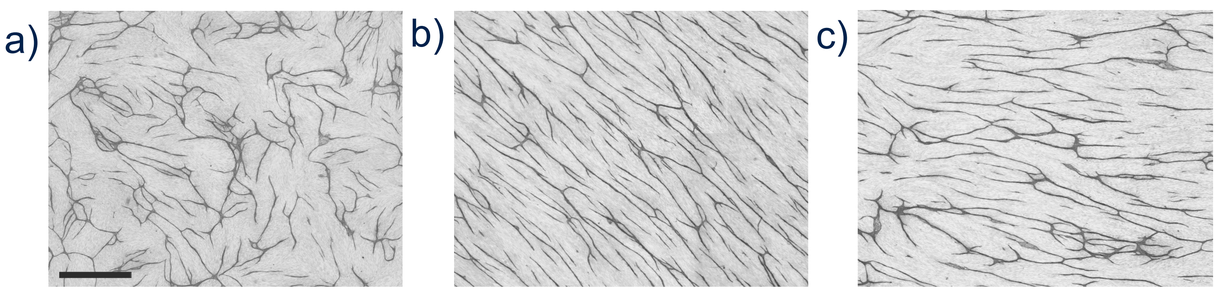

Figure 2: Network formation under 0 (a), 7.4% (b) and 13.2% (c) strains respectively (anti-PECAM-1 staining) from one experiment. Scale bar: 1mm

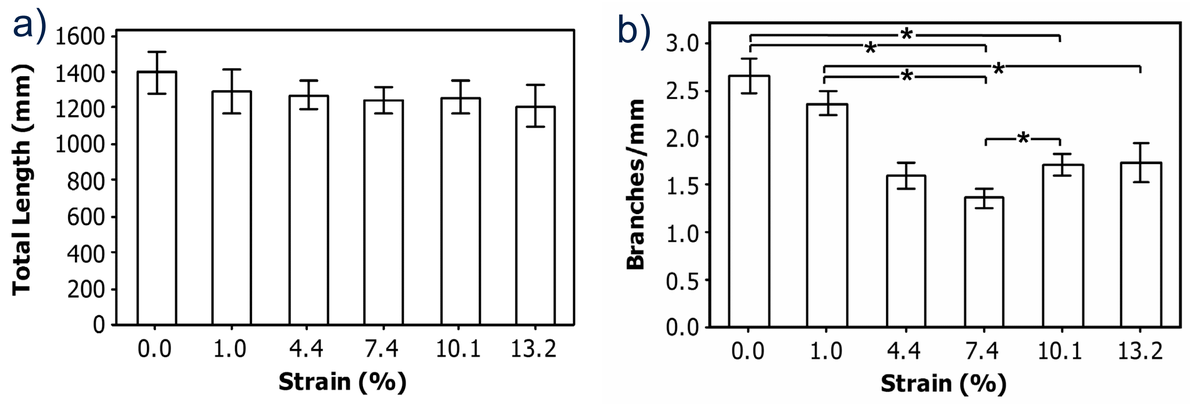

Figure 3: Sum of network lengths measured across 3 replicate samples (a); mean number of branch-points per mm "vessel" length (calculated over 3 replicates per experiment) (b); charts show means from 3 independent experiments ± standard deviations. *p<0.05, paired Student's t-test

"Tissue" vs "Vessel" Morphology (preliminary)

Endothelial network formation in "tissues" pre-cultured under stretched and static conditions:

- 1st phase (3 days): "tissue-building" (fibroblast culture);
- 2nd phase (3 days): network formation (co-culture).

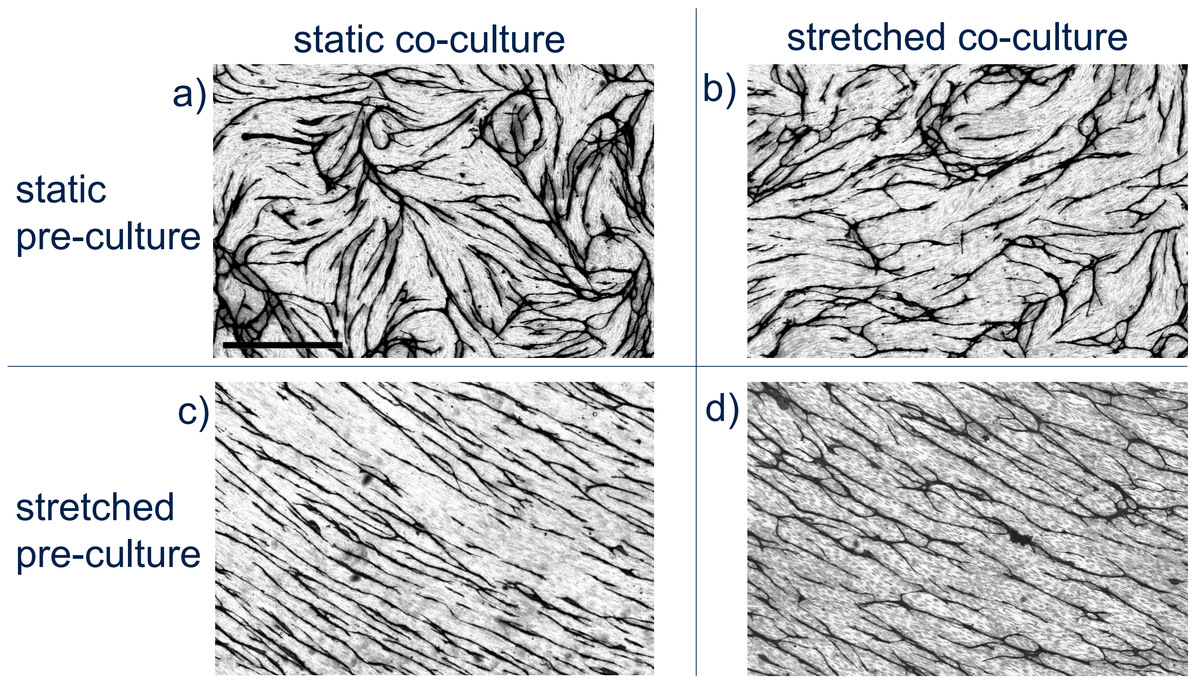

Figure 4: Fibroblasts cultured for 3 days in static (a, b) or cyclic strain (c, d) conditions; after seeding HUVECs, samples cultured a further 3 days with (b, d) or without (a, c) cyclic stretching. Anti-PECAM-1-labelled HUVECs are shown at the peripheral region of the BioFlex membranes, where strain (where applied) is predominantly radial; alignment seen in (c) and (d) is circumferential, perpendicular to the maximum principal strain. Scale bar: 1mm

CONCLUSION

The organisation of new blood vessels under cyclic strain dominated by the structure of the supporting fibroblast tissue.

We propose that, in fracture healing, it may be the formation and integrity of the granulation tissue and callus that is ultimately critical in revascularisation and its failure under severe strain conditions.

ACKNOWLEDGEMENTS

This work was supported by the AO Research Fund of the AO Foundation (project no. S-08-87K) and the DFG GSC 203 Berlin-Brandenburg School for Regenerative Therapies.

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